# CellCycleFlowEx Kit

## 200 tests | Cat. No. ED7069

RUO

**Not for use in diagnostic or therapeutic procedures.**

## Technical Data Sheet (EN)

Version: ED7069_TDS_v4_EN

Date of Issue: 20-12-2024

Symbols used in the product labeling

| | | | |
|---|---|---|---|
| RUO | Research Use Only | | Keep Dry<br>Keep away from rain |
| | Manufacturer | | Caution |
| | Consult instructions for use | CONC 10× | Concentrated solution (10x) |
| | Contains sufficient for <n> tests | CONTENTS | Contents |
| REF | Catalogue number | | |
| LOT | Batch code | | |
| | Use by date | | |
| | Temperature limit | | |
| | Keep away from sunlight | | |

## Description

The product is For Research Use Only. Diagnostic or therapeutic applications are strictly forbidden.

The CellCycleFlowEx Kit is intended for cell cycle analysis using flow cytometry and is suitable for testing suspensions of isolated cells, such as leukocytes isolated from peripheral blood (PBMC) or cells from tissue culture.

DNA during the cell cycle, proliferating cells duplicate their DNA, which is then equally segregated into daughter cells. Measurement of cellular DNA content within a proliferating cell population enables identification of cells in specific cell cycle phases:

- G0/G1 phase: Cells with DNA content equivalent to resting cells.
- S phase: Cells actively synthesizing their DNA.
- G2/M phase: Cells with double the DNA content of resting cells.

Such analysis helps assess cellular proliferation in response to stimulation with mitogens (lymphocyte transformation test) or to monitor effects of cell cycle inhibitors.

During the procedure, cells are washed and resuspended to create a uniform suspension. The cells are then fixed and permeabilized with 70% ethanol. Cellular DNA is stained with propidium iodide, a dye which passes through the permeabilized membrane and intercalates between the bases of nucleic acids. Propidium iodide binds to the DNA stoichiometrically, meaning that the amount of dye bound is proportional to the amount of DNA within the cell. Staining occurs in the presence of RNase, which removes the interfering RNA. After dye excitation with laser in a flow cytometer, the stained cells emit fluorescence with an intensity proportional to their DNA content. The analysis distinguishes the cells in G0/G1, S and G2/M phase of the cell cycle based on the fluorescence intensity.

## Reagent(s) provided

### Contents

The product CellCycleFlowEx Kit is sufficient for 200 tests and is provided with the following reagents:

**RNase A ED7069-1** (2 vials) containing 1 ml of RNase A solution.

**Propidium Iodide ED7069-2** (1 vial) containing 2 ml of propidium iodide solution.

**Wash Buffer ED7069-3** (2 bottles) each containing 30 ml of concentrated (10X) solution.

## Materials required but not provided

Round bottom test tubes (12 x 75 mm)

70% ethanol, p.a. purity

## Equipment required

Automatic pipette with disposable tips (20 µl – 2 ml) for pipetting specimen and reagents

Vortex mixer

Ice bath (ice cubes or crushed ice)

Centrifuge

Flow cytometer with blue laser excitation 488 nm, detectors for scattered light, optical filters and emission detectors appropriate to collect signals from fluorochromes provided in Table 2.

**Table 2** Spectral characteristic of fluorochromes use in the product

| Flurochrome | Excitation [nm] | Emission [nm] |
|---|---|---|
| Propidium iodide | 488 | 617 |

## Storage and handling

CellCycleFlowEx Kit consists of two parts:

- Box containing Wash Buffer and Propidium iodide. Store at 2-8 °C.
- Resealable plastic bag containing RNase A. Store at -20 °C.

Avoid prolonged exposure to light.

See Section Procedure (Preparation of reagent(s) provided) for information about the storage conditions and stability of working solutions (where applicable).

## Warnings, precautions and limitations of use

### GHS Hazard Classification

Consult Safety Data Sheet (SDS) available on the product page at www.exbio.cz for the full information on the risks posed by chemical substances and mixtures contained in the Product and how they should be handled and disposed.

### Biological Hazard

Human biological samples and blood specimens and any materials coming into contact with them are always considered as infectious materials.

Use personal protective and safety equipment to avoid contact with skin, eyes and mucous membranes.

Follow all applicable laws, regulations and procedures for handling and disposing of

infectious materials.

### Evidence of deterioration

Normal appearance of Wash Buffer is a clear liquid. If precipitation is observed, bring the solution to room temperature and allow the precipitate to dissolve before use.

Normal appearance of Propidium Iodide is a red-orange colored liquid. Do not use the reagent if you observe any change in appearance, for example turbidity or signs of precipitation.

Normal appearance of RNAse A is a clear liquid. Do not use the reagent if you observe any change in appearance, for example turbidity or signs of precipitation.

### Limitation of use

Do not use after the expiry date stated on the product labels.

## Specimen

The kit is suitable for testing of cells in suspension, such as leukocytes isolated from peripheral blood (PBMC) or cells from tissue culture.

## Procedure

### Preparation of reagent(s) provided

Wash Buffer

The reagent is 10X concentrated and must be diluted with deionized water prior use (1 volume of the concentrated solution and 9 volumes of deionized water). Dilute Following the first opening, the reagent retains its performance characteristics until the expiry date when stored under the stated conditions in its original primary container.

The diluted Wash buffer (1X) is stable for 6 months when stored in a liquid dispenser or closed container at 2-8 °C.

> **CAUTION:** Wash Buffer (10X) may contain precipitated salts. If present, place the bottle to room temperature or into a water bath set to 37 °C and wait untill the salts dissolve. Mix to ensure homogeneity before use.

DNA staining solution

Prepare DNA staining solution according to the following table. A minimum of 0.5 ml is needed per a test tube. The DNA staining solution is stable for 2 weeks when stored stored in a liquid dispenser or closed container at 2-8 °C.

| | |
|---|---|
| Wash Buffer (1X) | 1 ml |
| Propidium Iodide | 0.02 ml |
| RNase A | 0.02 ml |

## Preparation of reagent(s) not provided

70% ethanol

Prepare 70% ethanol (not provided) by diluting either absolute ethanol or 96% ethanol in deionized water. A minimum of 2.0 ml is needed per a test tube. Store in a liquid dispenser or closed container at -20 °C (-15 °C to -30 °C).

## Sample wash before staining

1. Centrifuge the cells for 5 min at 300× g at laboratory temperature, discard the supernatant into a container with an appropriate disinfectant.
2. Add 1 ml of cold (2-8 °C) diluted (1X) Wash Buffer. Resuspend the cells using a vortex mixer.
3. Centrifuge the cells for 5 min at 300× g at laboratory temperature, discard the supernatant into a container with an appropriate disinfectant.
4. Resuspends the cells in cold (2-8 °C) diluted (1X) Wash Buffer to concentration 2.5-10 x $10^6$ cells / ml.

**NOTICE:** A minimum of 200 µl is needed per a test tube.

## Sample fixation with ethanol

1. Prepare round bottom test tubes (12 × 75 mm) and label them according to the specimen ID and/or according to the stimulation conditions.
2. Pipette 200 µl of the cell suspension to the bottom of the test tube and place the tube on ice.
3. Add dropwise 2 ml of cold 70% ethanol (-15 to -30 °C) while continuously mixing (vortexing) the tube. Then immediately place the tube back to ice.

**NOTICE:** The procedure may be stopped at this step. The fixed cells can be transported or stored at 2-8 °C for up to 2 weeks.

## Sample staining

1. Centrifuge the cells for 5 min at 300×g at laboratory temperature, discard the supernatant into a container with an appropriate disinfectant.
2. Add 1 ml of cold (2-8 °C) diluted (1X) Wash Buffer. Resuspend the cells using a vortex mixer.
3. Centrifuge the cells for 5 min at 300×g at laboratory temperature, discard the supernatant into a container with an appropriate disinfectant.
4. Add 0.5 ml of DNA staining solution. Resuspend the cells using a vortex mixer.
5. Incubate for 30 minutes at room temperature in the dark.

6. Acquire the stained sample immediately using flow cytometer. If the stained sample will not be acquired immediatelly, store it at 2-8 °C in the dark and analyze within 4 hours.

   **CAUTION:** Cells in the DNA staining solution may form aggregates in time. These must be resuspended by vortexing before flow cytometric analysis.

   **NOTICE:** DNA staining continues if the processed sample is stored. Therefore, samples measured in 30 minutes and those measured after 4 hours may show differences in their G1/G2 peak positions. The mean fluorescence intensity will increase by 10%, the G2/G1 fluorescence ratio will also be higher.

## Flow cytometry analysis

The flow cytometer selected for use with the product CellCycleFlowEx Kit shall be calibrated on a routine basis using fluorescent microbeads to ensure stable sensitivity of detectors according to the cytometer manufacturers instructions.

If not maintained properly the flow cytometer may produce false results.

Refer to the manufacturer's cytometer specifications for lasers and fluorescence detectors according to the excitation and emission characteristics of the fluorochromes in Section Equipment required.

Set voltages on the fluorescence detectors of interest prior to stained specimen analysis. Voltage on a PMT detector should be set high enough, so that minimum of negatively stained events interfere with 0th channel on the fluorescence axis. Also, PMT detector voltage should not exceed values at which positive events are pressed to the right axis.

Compensate fluorescence signals between detectors prior to or after data acquisition. Data may be incorrectly interpreted if fluorescence signals are compensated improperly or if gates are positioned inaccurately.

For measured data analysis it is possible to use cytometer software developed by the manufacturer, or software dedicated for offline cytometry data analysis (for example FlowJo™, VenturiOne®, Infinicyt™).

Acquire at least 20,000 events per sample. For precise measurements we recommend setting the flow rate too the lowest setting, as high acquisition speeds can negatively affect the resolution of the populations.

There is a risk of misinterpreting the data if the singlet gate and boundaries for the G0/G1, S and G2/M phase are not correctly placed.

## Data analysis

Visualize the measured data in the side-scatter (SSC) versus forward scatter (FSC) plot. Set a gate around the target cell population (Figure 1A, 1B).

**Figure 1A** Delineation of the target cell population.
(PBMCs stimulated in vitro with phytohemagglutinin at 5 µg/ml for 3 days)

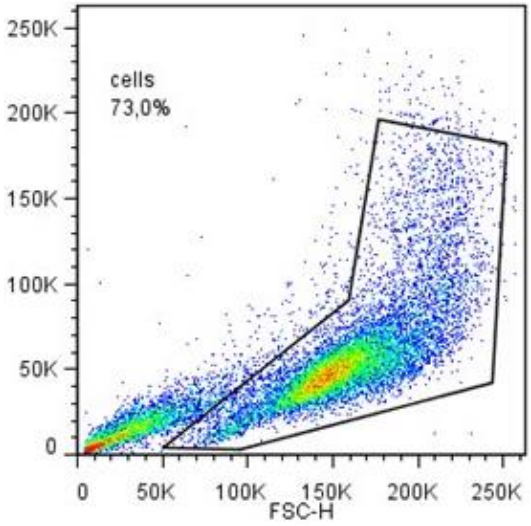
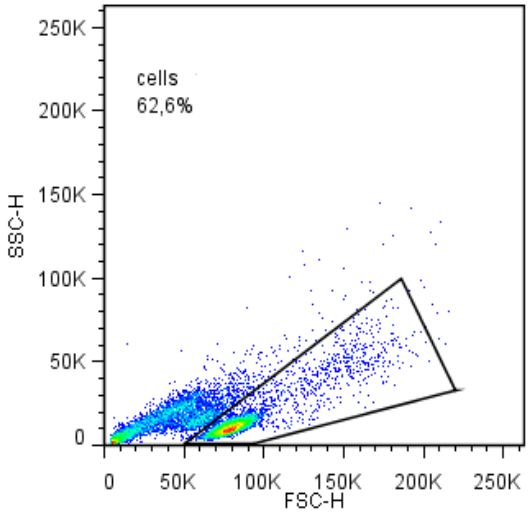


**Figure 1B** Delineation of the target cell population.
(PBMCs cultured in vitro without stimulation for 3 days)



Visualize the target population in the PE-Width (PE-W) versus PE-Area (PE-A). Set the gate for singlets (Figure 2A, 2B).

**Figure 2A** Delineation of singlets.
(PBMCs stimulated in vitro with phytohemagglutinin at 5 µg/ml for 3 days)

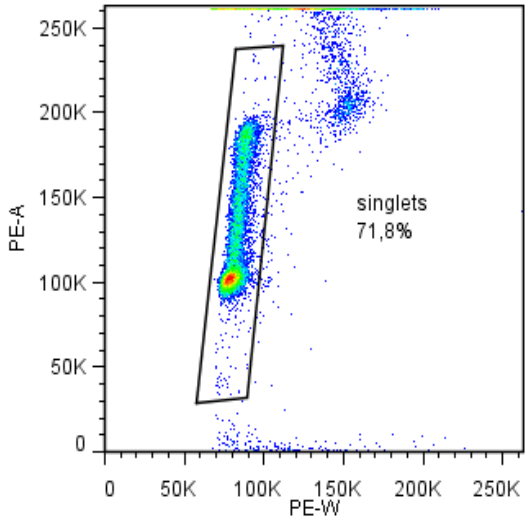
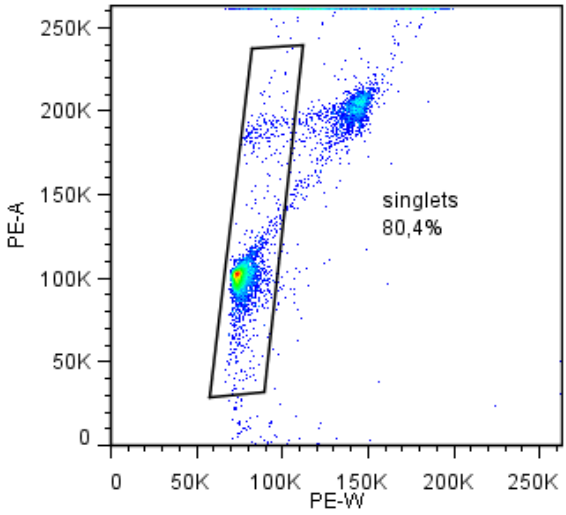


**Figure 2B** Delineation of singlets.
(PBMCs cultured in vitro without stimulation for 3 days)



Visualize singlets as a histogram, where the X axis represents the fluorescence intensity in the PE detector. Set the axis to linear scaling, and adjust the PMT to ensure the G1 peak is positioned at one third of the scale (Figure 3A, 3B).

Set the boundaries between the G0/G1, S and G2/M phases of cell cycle, then calculate the proportion of cells in each phase. Alternatively, use suitable software (e.g. FlowJo) that offers algorithms to automatically determine the distribution across the cell cycle phases.

**Figure 3A** Distribution of fluorescence intensity signal according to the cellular DNA content, with boundaries set between the G0/G1, S, and G2 / M phases of the cell cycle. (PBMCs stimulated in vitro with phytohemagglutinin at 5 µg/ml for 3 days)

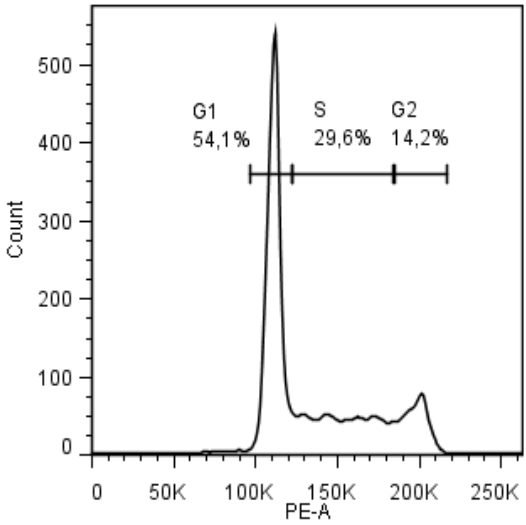
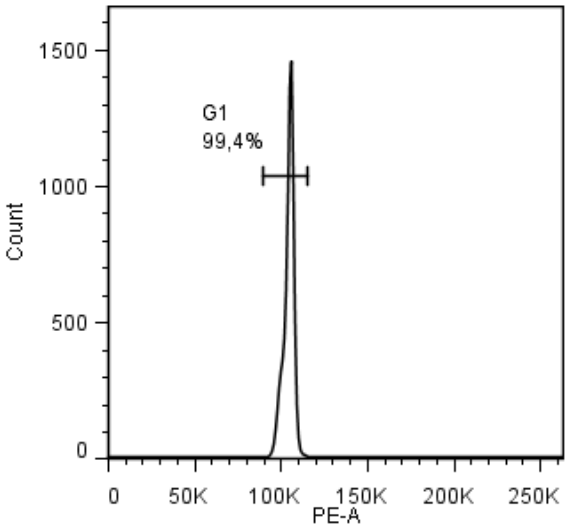


**Figure 3B** Distribution of fluorescence intensity signal according to the cellular DNA content, with boundaries set between the G0/G1, S and G2 / M phases of the cell cycle. (PBMCs cultured in vitro without stimulation for 3 days)



## References

1) Pozarowski P, Darzynkiewicz Z. Analysis of cell cycle by flow cytometry. Methods Mol Biol. 2004;281:301-11.

## Use of Third Party Trademarks

FlowJo™ is registered trademark of Becton, Dickinson and Company, VenturiOne® is a registered trademark of Applied Cytometry, Infinicyt™ is a registered trademark of Cytognos S.L..

## Revision History

Version 4, ED7069_TDS_v4

TDS layout has been changed. Includes updates to the kit's technical specifications, reagent preparation, and storage instructions. Additionally, new warnings regarding potential hazards and fluorescence calibration have been added, along with revised terminology in the reagent descriptions and updated guidelines for data analysis.

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**NOTICE:** Any serious incident that has occured in relation to the product shall be reported to the manufacturer and the local competent authority.